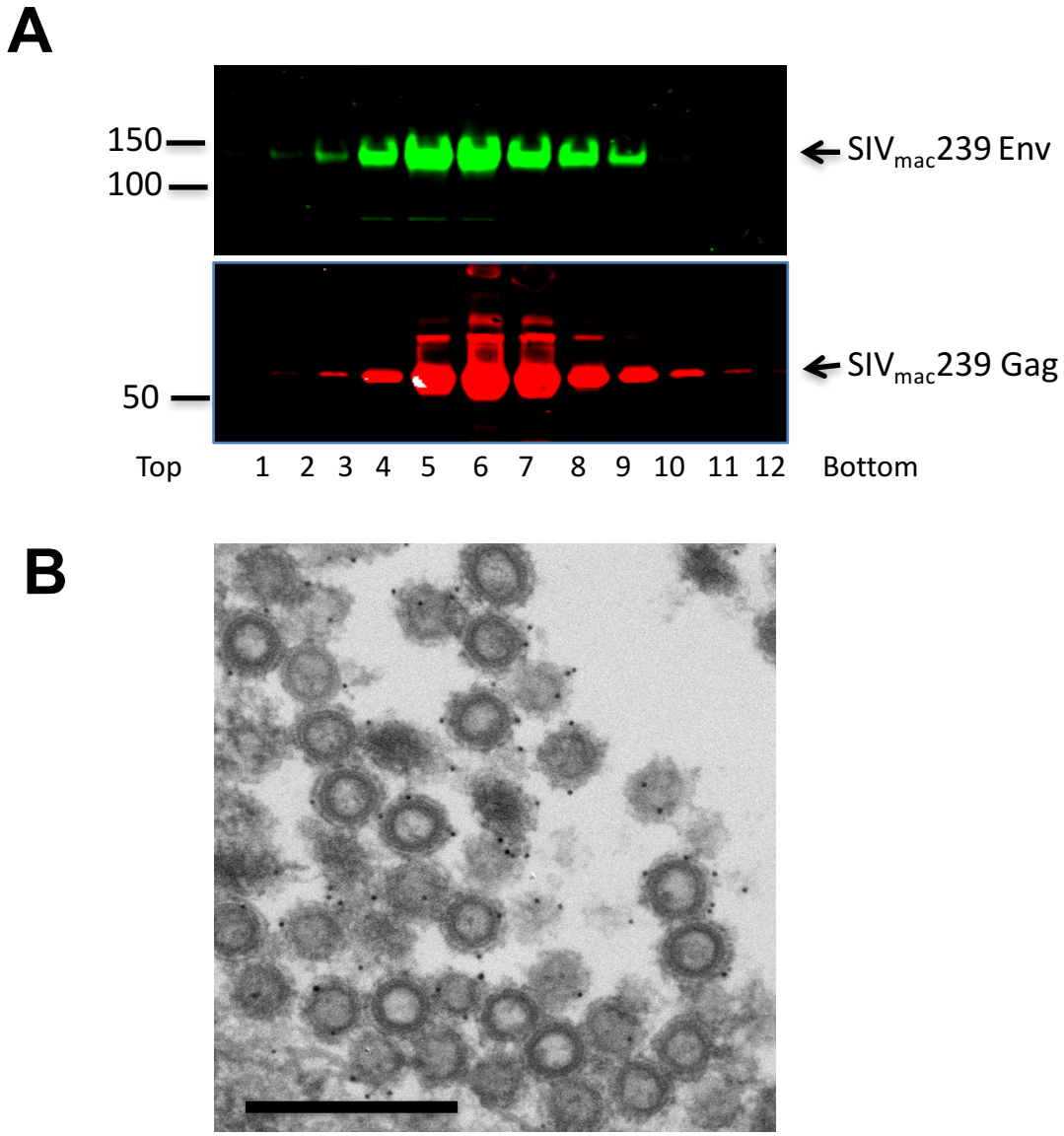
Supplementary Figures

Supplementary Table 1

		Age	A01	B08	B17	Allele Phenotype
VLP +Alum						
	RKu9	9	-	-	-	High- TFP/CYP
	RVo8	11	-	-	+	High- TFP/CYP
	RWt7	12	-	+	-	High- TFP/TFP
	RCv5	16	-	-	-	High-TFP/TFP
	RAc6	15	+	+	-	Moderate-Q/TFP
	RPk8	11	+	-	-	Moderate-Q/CYP
	RCl8	11	+	-	-	Moderate- Q/CYP
	RCj7	12	-	-	-	Moderate- Q/TFP
VLP + PLGA(MPL+R848)						
	RRu5	16	-	-	-	High-TFP/TFP
	RCb6	15	-	-	-	High-TFP/TFP
	RQf6	15	-	-	-	High-TFP/TFP
	RBh9	10	-	+	-	High-TFP/CYP
	REe8	11	-	-	-	High-TFP/CYP
	RKf10	8	-	—		High- CYP/CYP
	ROk9	10	+	-	+	Moderate-Q/CYP
	RJe8	11	+	-	-	Moderate-Q/TFP
	Rlj9	10	+	-	-	Moderate-Q/TFP
SIVgp140 +SIVp55 gag + Alum						
	RSe8		-	-	-	High-TFP/TFP
	RZc5	17	-	-	+	High-TFP/TFP
	RVd8	11	-	-	-	Moderate-Q/TFP
	RNe8	13	-	+	-	Moderate- Q/CYP
	RIc4	19	-	-		Moderate- Q/TFP
	RWl5	17	-	—		High- TFP/TFP
	RWv8	10	+	-	-	High- TFP/TFP
	RKa7	13	+	-	-	High- CYP/CYP
	RNf7	11	+	—		High- TFP/TFP
	RJg4	19	-	-	-	Moderate- Q/TFP
SIVgp140 +SIVp55 gag + PLGA(MPL+R848)						
	RQm7	12	-	-	-	High- TFP/TFP
	RHe4	19	-	+		High- TFP/TFP
	RQq8	11	-	-	-	High- CYP/CYP
	RPs6	14	-	—		Moderate- Q/CYP
	RSp9	9	-	—		Moderate- Q/TFP
	RMv5	16	-	-	-	Moderate- Q/CYP
	RSb9	10	+	-	-	High- TFP/TFP
	RAj9	10	+	-	-	High- TFP/TFP
	RUj5	17	+	-	-	High- TFP/TFP
	RMj7	12	—	+	-	Moderate- Q/TFP

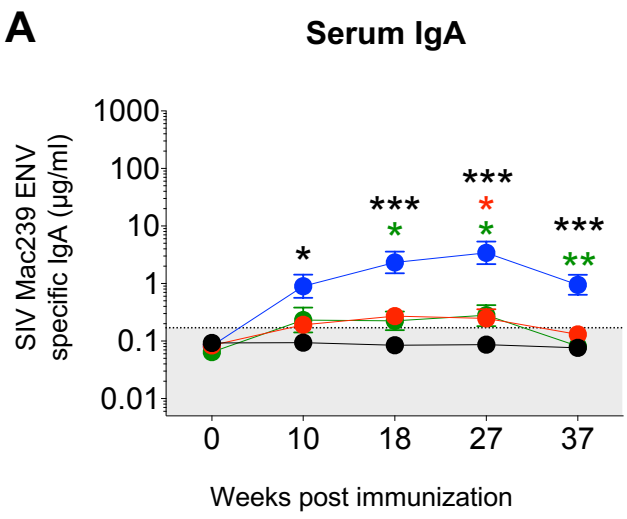
Supplementary Table 1:
Table lists experimental group assignment, ID, age, MHC alleles (Mamu A*001, B*008 and B*017 status) as well TRIM5α allele genotyping of the animals used in the study. *Animals infected upon intra-vaginal challenges are highlighted in red*.

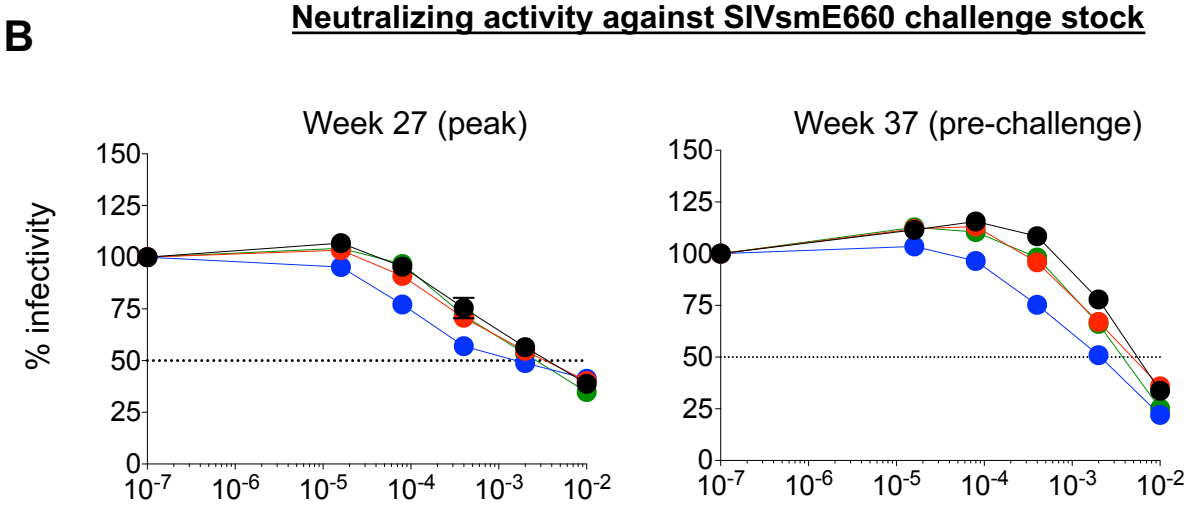
Supplementary Figure 1



Supplementary Figure 1:
Characterization of SIVmac239 VLPs. A) VLPs were subjected to equilibrium density centrifugation on a 20-60% gradient. Equal fractions were collected, concentrated, and analyzed by Western blotting and detection using a LiCor Odyssey instrument. B) Transmission electron microscopic image of SIVmac239 VLPs, marked by immunogold labeling using anti-SIV Env antisera. Bar = 0.5 μm

Supplementary figure 2



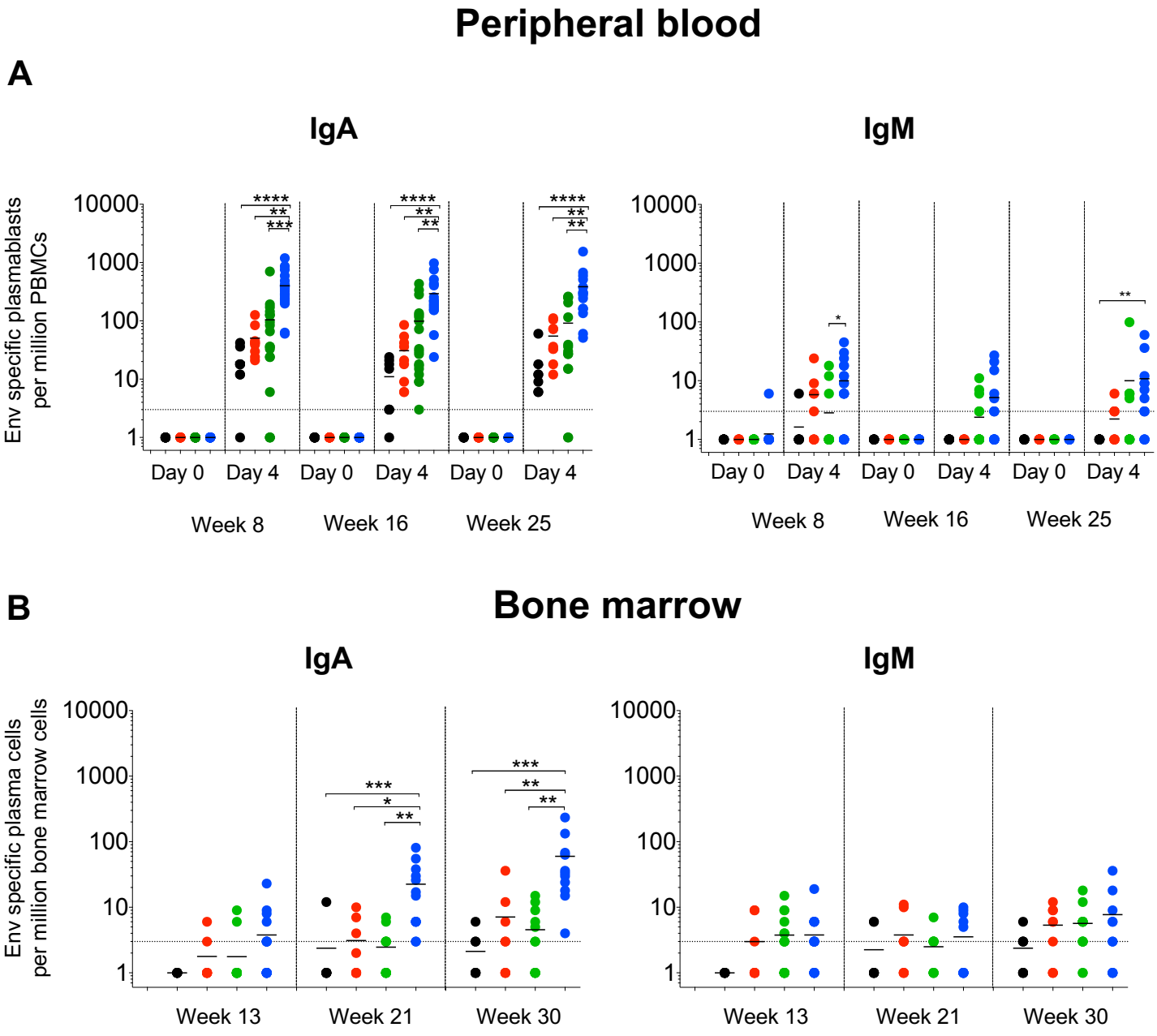


Supplementary Figure 2:
Antibody responses in RMs immunized with VLP or Protein antigens with alum or NP adjuvants

A) SIVmac239 derived recombinant gp140 Env-specific IgA binding antibody responses were assayed in serum at time points indicated in the graph. Binding titers are represented in μg/ml and color coded circles indicate geometric mean ± standard error of ~8-10 animals/treatment groups.

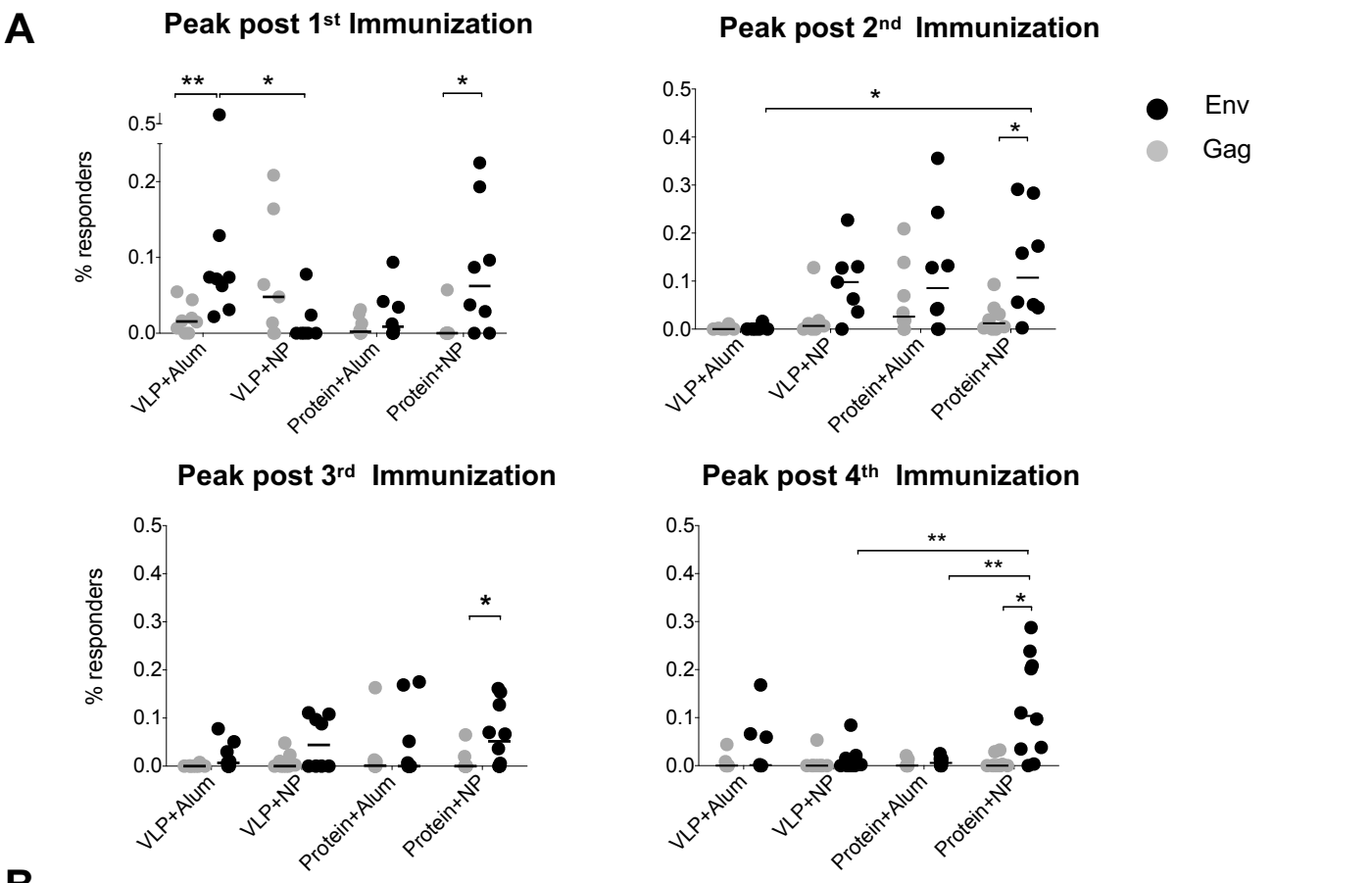
B) Virus infectivity curves depict neutralization activity against the SIVsmE660 challenge stock (swarm) specific neutralizing activity in serum was measured as week 27 (peak time point post final immunization) and at week 37(4 weeks prior to challenge).

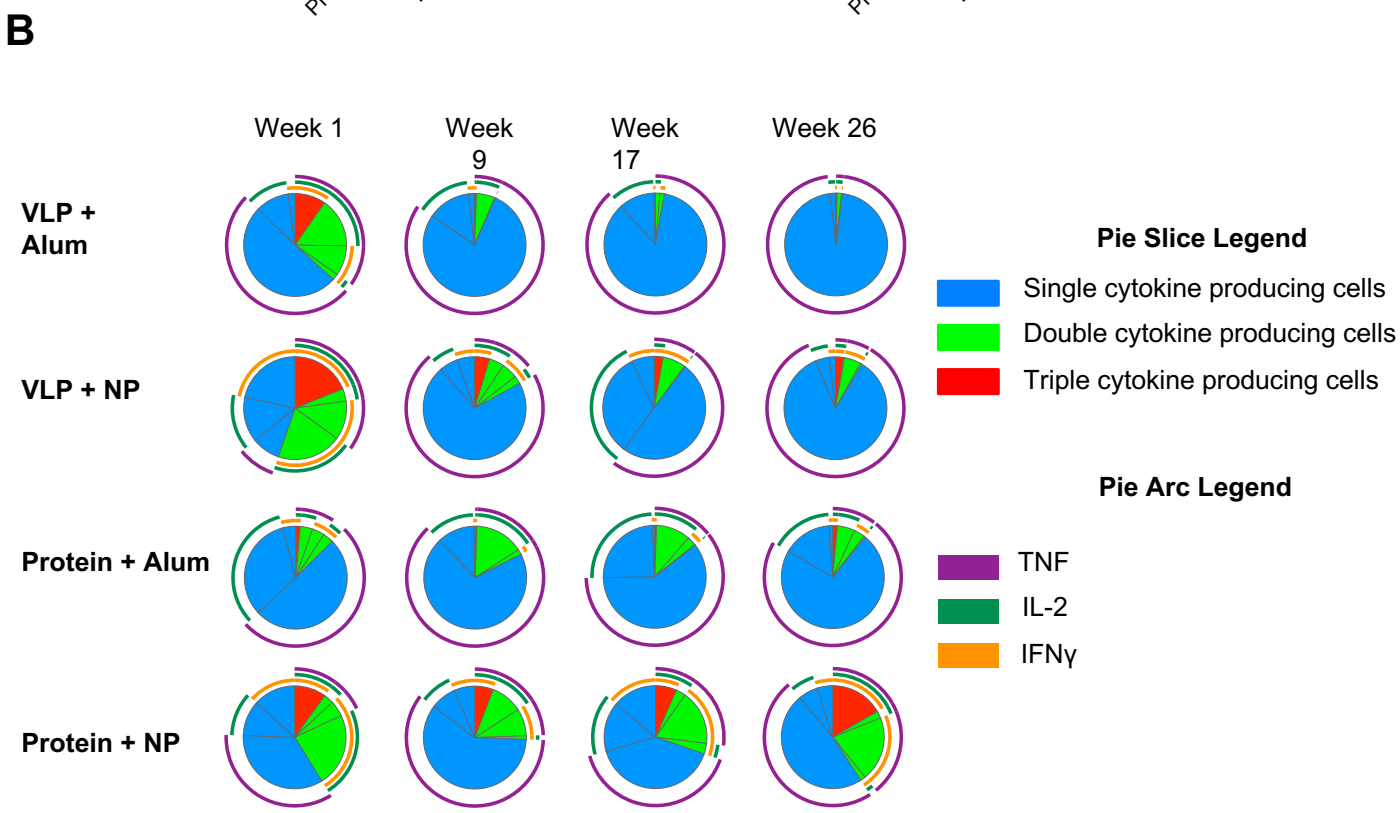
Supplementary Figure 3



Supplementary Figure 3:
NP adjuvant in the presence of protein antigen induces significantly higher SIV Env-specific IgA and IgM secreting plasmablast response in peripheral blood and plasma cell response in the bone marrow in RMs.
A) Magnitude of Env-specific IgA (left) and IgM (right) secreting plasmablast responses at day 4 after each round of boost immunization in all treatment groups are represented in the graph-using scatter plots. B) Magnitude of Env-specific IgA (left) and IgM (right) secreting plasma cells in the bone marrow at 5 weeks post each round of boost immunization in all treatment groups are represented in the graph using scatter plots. Horizontal bars represent median responses. Statistical significance for difference in magnitude of response between treatment groups in all graphs was evaluated using a multigroup comparison, Kruskal-Wallis test followed by Dunn's correction. **** Indicates a p value <0.0001, *** indicates a p value <0.001, ** indicates a p value <0.01 and * indicates a p value <0.05.

Supplementary Figure 4



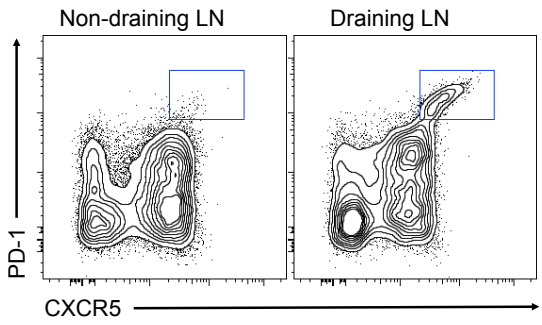


Supplementary Figure 4:
NP adjuvant in the presence of protein antigen induces significantly higher Env-specific CD4+ T cell responses in peripheral blood.
Env and gag specific CD4 + T cell responses were evaluated using ex vivo stimulation with consensus Env and Gag specific peptide pools followed by intracellular cytokine staining. A) Env and Gag specific CD4+ T cell responses a week after each round of immunization are represented as frequencies of cytokine (IFN-γ, TNF or IL-2) producing cells as a percentage of non-naïve CD4+ T cells as described before. Scatter plots show each animal assayed with horizontal bars represent median responses in each treatment group. Env and Gag responses are highlighted in black and gray shades respectively.
B) Polyfunctionality of cytokine producing cells was determined using the SPICE and PESTLE software (NIH). Single, double or triple cytokine producing cells are color coded in pie slices as indicated in the legend. Color-coded external arcs represent overlapping production of cytokines IFN-γ, TNF and IL-2. Statistical significance for difference in magnitude of response between treatment groups in all graphs was evaluated using a multigroup comparison, Kruskal-Wallis test followed by Dunn's correction. **** Indicates a p value <0.0001, *** indicates a p value <0.001, ** indicates a p value <0.01 and * indicates a p value <0.05. Significance of difference in magnitude between Env and gag specific response was inferred using a Mann-Whitney test.

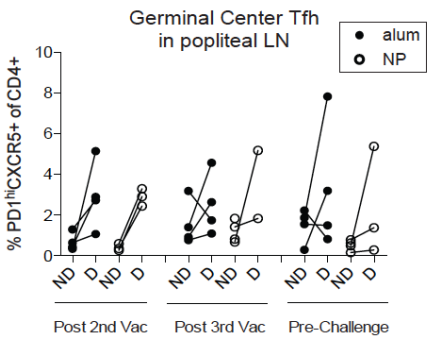
Supplementary Figure 5

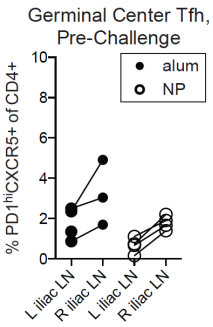
Tfh Responses

A **Gated on CD4+ T cells**



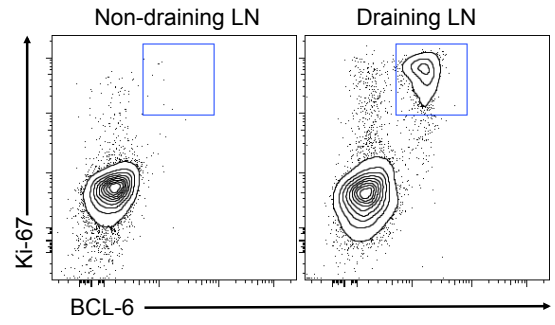
B



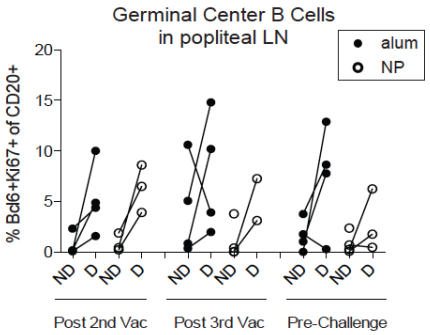


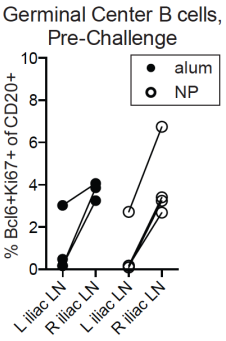
Germinal center B cell Responses

C **Gated on CD20+ B cells**



D





Supplementary Figure 5:
Follicular t helper cell and germinal center responses in draining and non-draining lymphoid organs were evaluated by flow cytometry.

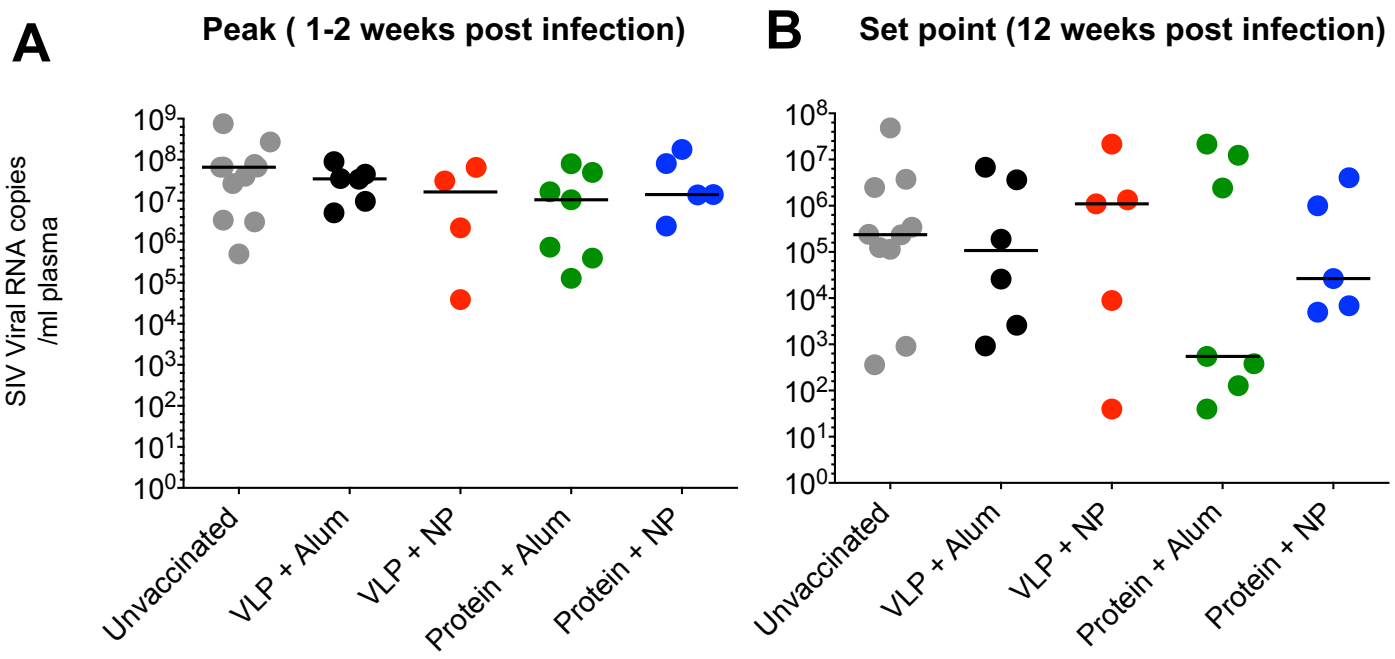
A) Representative flow cytometry plots show the presence of higher frequencies of GC Tfh cells in draining popliteal LNs post immunization in comparison with frequencies of GC Tfh cells in non-draining LNs.

B) Dot plots indicate increased frequencies of GC Tfh cells in draining popliteal LNs in comparison with non-draining popliteal LNs post 2nd, 3rd and 4th immunization and Iliac LNs only after the 4th immunization in the study.

C) Flow cytometry plot shows Ki67+Bcl-6+ CD20+CD3- B cells that are identified as germinal center B cells.

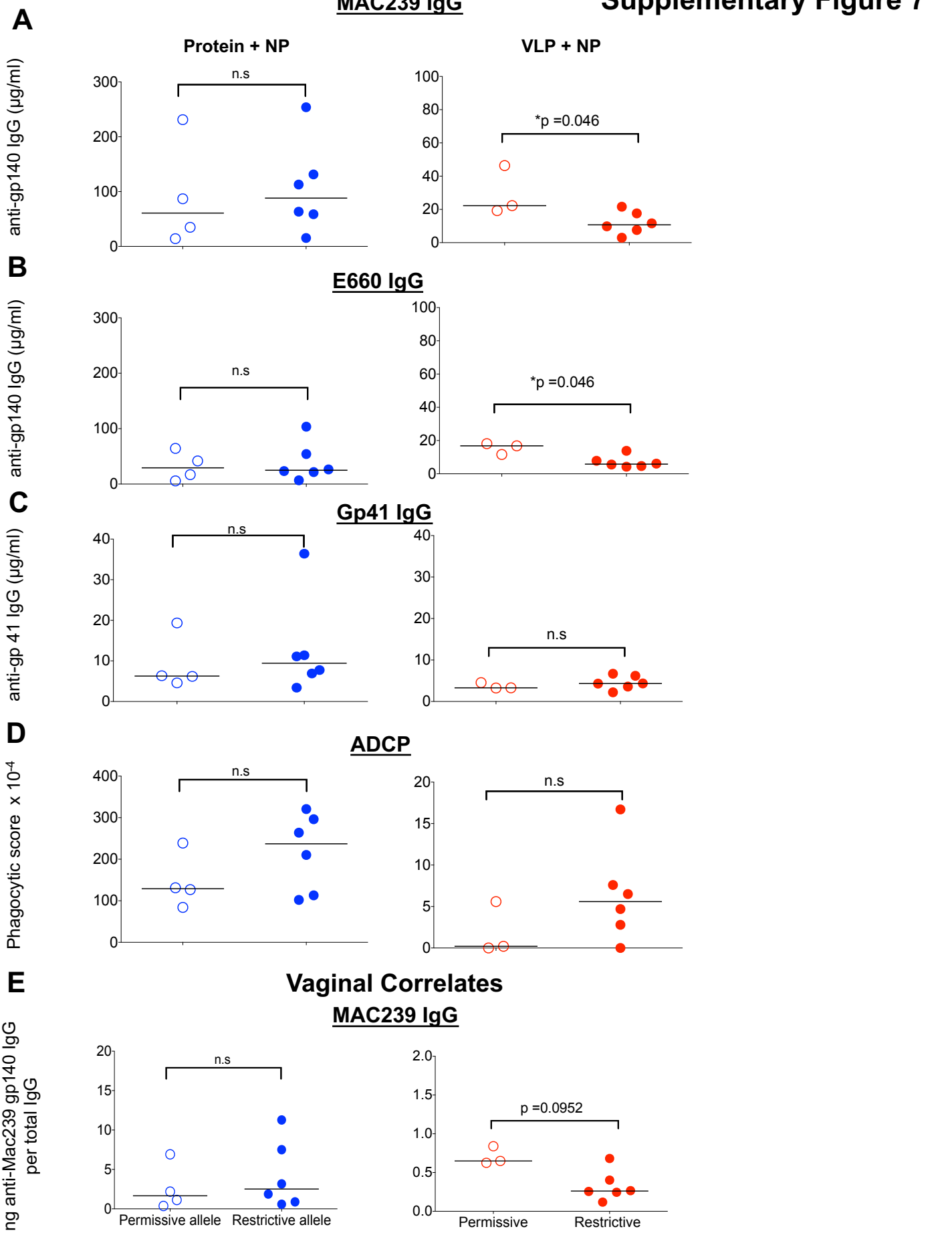
D) Dot plots indicate increased frequencies of GC b cells in draining popliteal LNs and Iliac LNs in comparison with non-draining LNs in the study.

Supplementary Figure 6



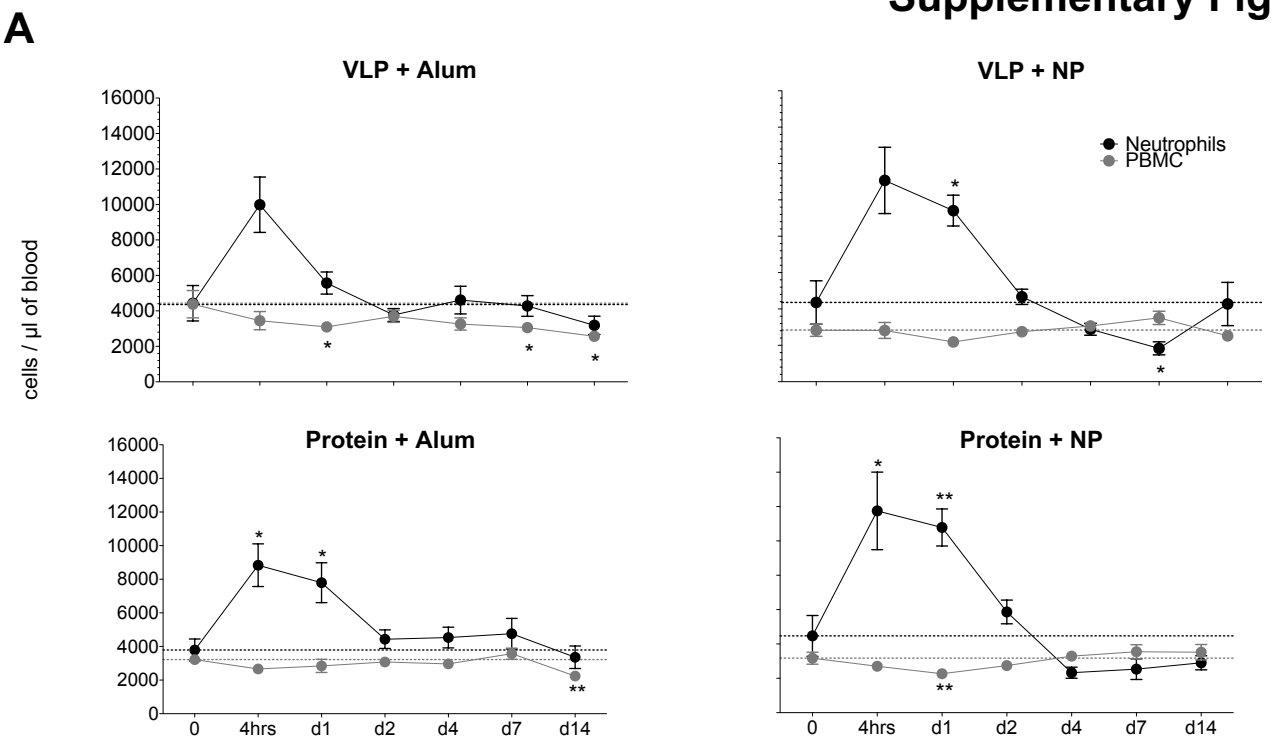
Supplementary Figure 6:
Peak and set point viral load in infected animals.
A) Scatter plots represent peak viral loads (1-2) weeks post infection in challenged animals. B) Scatter plots represent set point viral loads 12 weeks post infection in challenged animals. Horizontal bars indicate median values.

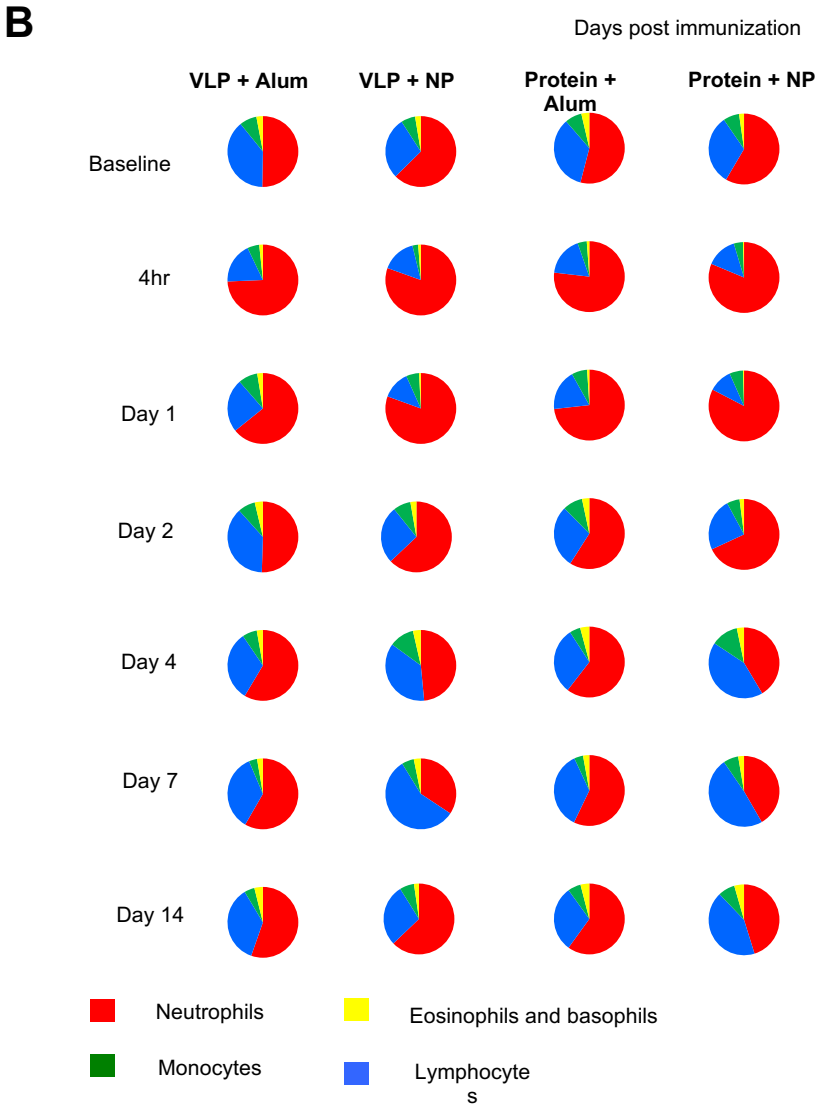
Supplementary Figure 7



Supplementary Figure 7:
Comparison of antibody responses in animals carrying TRIM5α restrictive and permissive alleles.
Graphs display SIVmac239 Env specific binding antibody responses in animals with restrictive and permissive alleles when immunized with VLP or Protein immunogen in the presence of NP adjuvant.

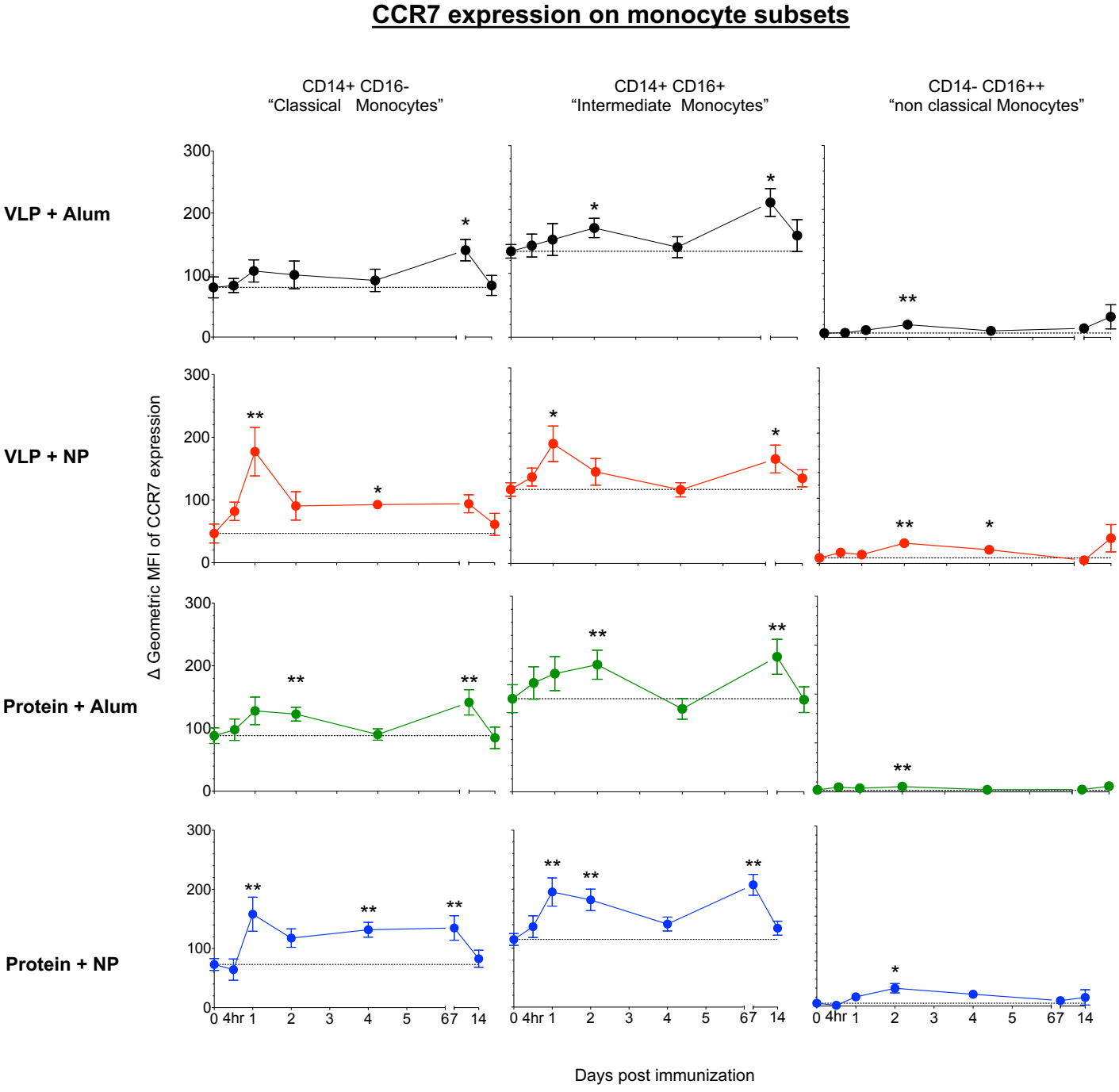
Supplementary Figure 8





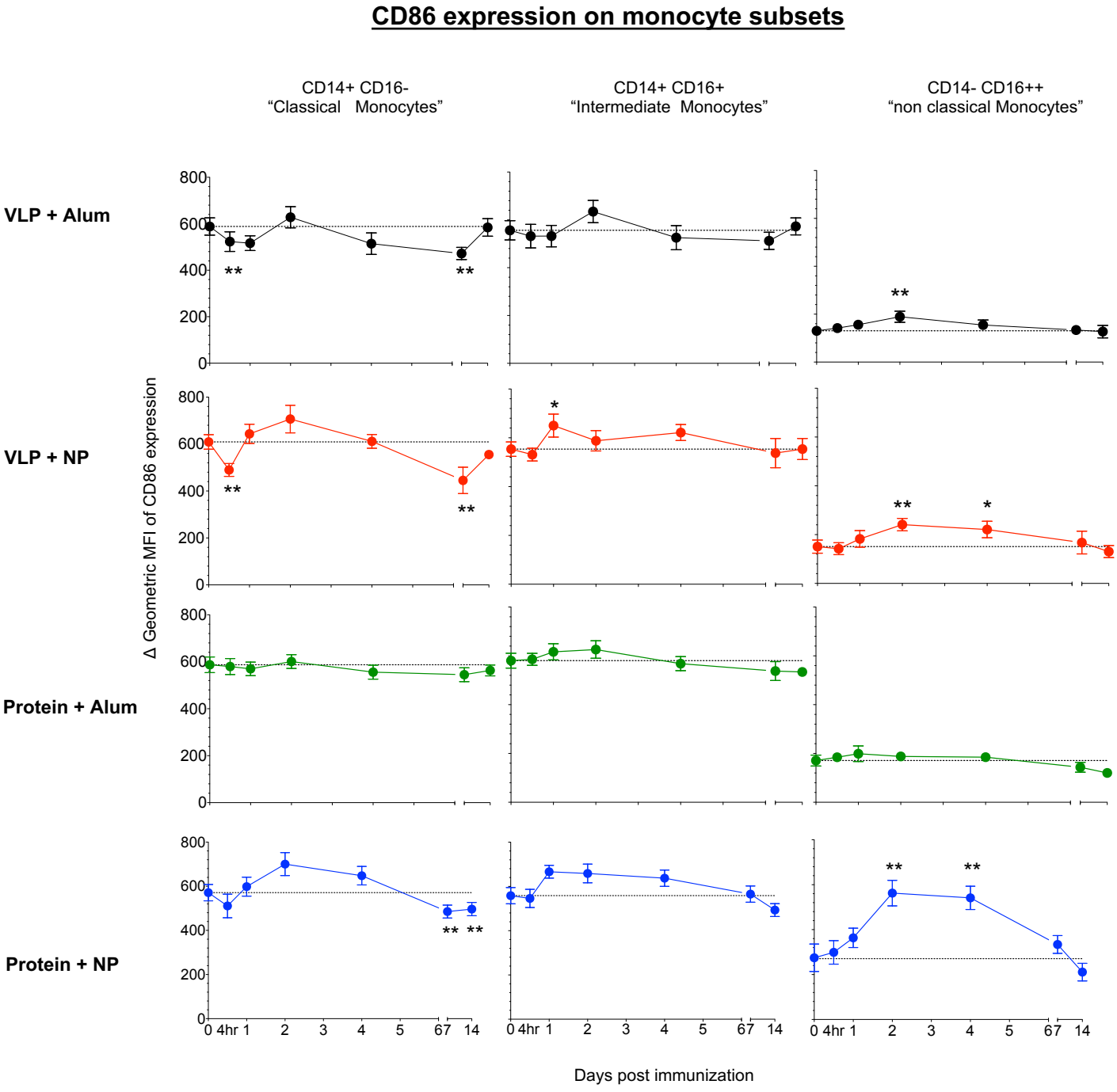
Supplementary Figure 8:
NP and Alum adjuvants induce a transient increase in neutrophils and minimal changes in total number of PBMCs in peripheral blood post primary immunization.
A) Kinetics of total numbers of neutrophils (black) and PBMCs (grey) in blood were assessed within the total WBCs by hematologic analysis. The PBMC subset represents pooled numbers of lymphocytes and monocytes. Statistical analysis of cell kinetics indicates significant change relevant to the baseline. Dotted lines indicate mean baseline frequencies of cell subsets in animals allocated to each treatment group. Statistical significance of change in frequencies of cell subsets in comparison with baseline was performed using a non-parametric Wilcoxon matched-pairs signed rank test. Circles at each time point in line graphs indicate mean ± sem.
B) Fractions of neutrophils (red), lymphocytes (blue), monocytes (green), and basophils and eosinophils (yellow) were measured. Each pie chart shows the mean percentage of the indicated cell populations within the experimental group at the indicated time points.

Supplementary Figure 9



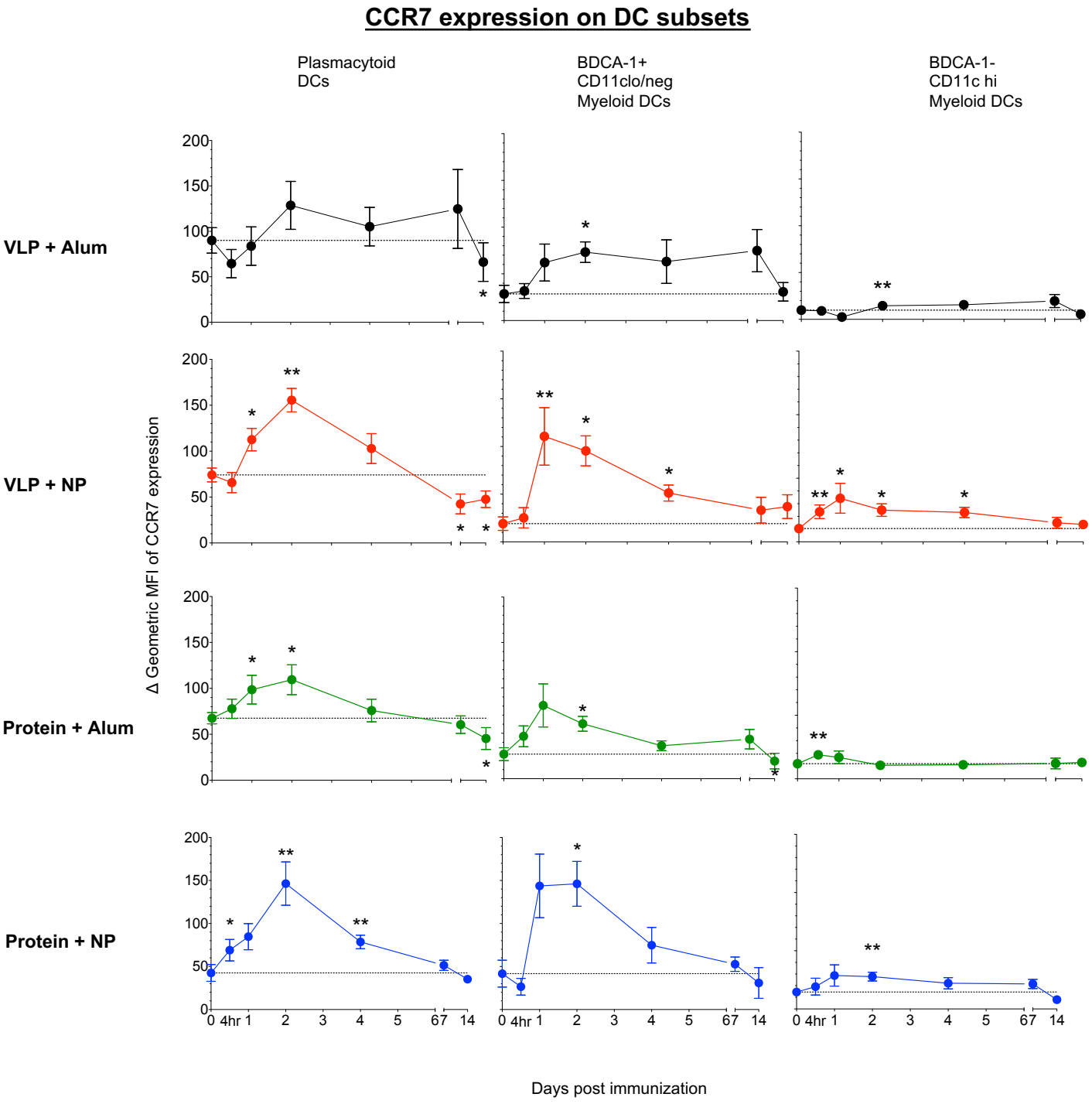
Supplementary Figure 9:
CCR7 expression on monocyte subsets in peripheral blood
Graphs indicate changes in expression levels of surface CCR7 on monocyte subsets in peripheral blood after primary immunization. Expressional levels are represented as geometric mean fluorescent intensity (Δ GEO MFI = geometric mean fluorescent signal with anti CCR7 antibody – geometric mean fluorescent signal with isotype stain) Statistical significance of changes in expression levels indicates significant change relevant to the baseline using a non-parametric Wilcoxon matched-pairs signed rank test. Dotted lines indicate mean baseline frequencies of cell subsets in animals allocated to each treatment group.

Supplementary Figure 10



Supplementary Figure 10:
CD86 expression on various monocyte subsets in peripheral blood
Graphs indicate changes in expression levels of surface CD86 on monocyte subsets in peripheral blood after primary immunization. Expressional levels are represented as geometric mean fluorescent intensity (Δ GEO MFI = geometric mean fluorescent signal with anti CD86 antibody – geometric mean fluorescent signal with isotype stain) Statistical significance of changes in expression levels indicates significant change relevant to the baseline using a non-parametric Wilcoxon matched-pairs signed rank test. Dotted lines indicate mean baseline frequencies of cell subsets in animals allocated to each treatment group.

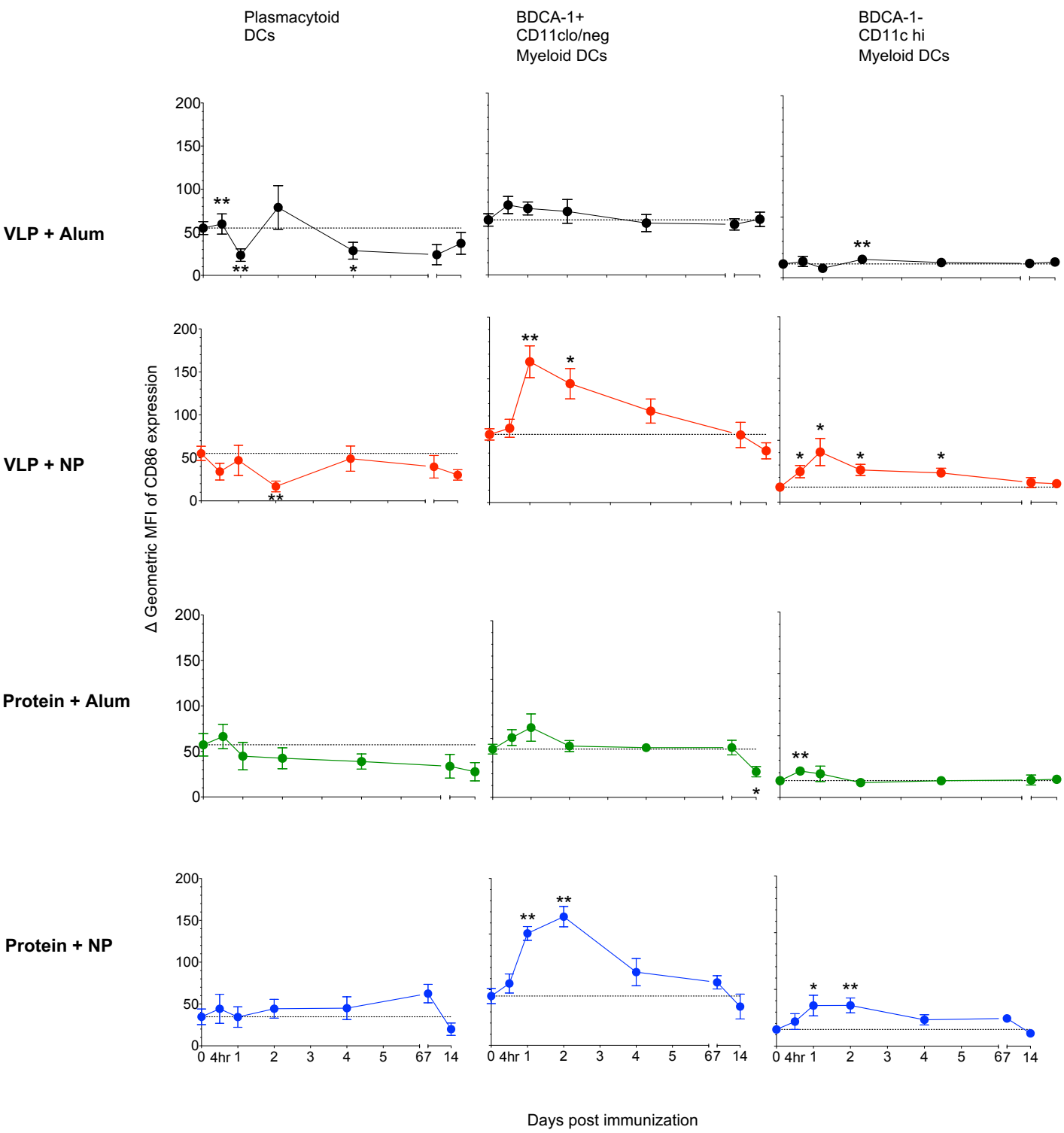
Supplementary Figure 11



Supplementary Figure 11:
CCR7 expression on DC subsets in peripheral blood

Graphs indicate changes in expression levels of surface CCR7 on monocyte subsets in peripheral blood after primary immunization. Expressional levels are represented as geometric mean fluorescent intensity (Δ GEO MFI = geometric mean fluorescent signal with anti CCR7 antibody – geometric mean fluorescent signal with isotype stain) Statistical significance of changes in expression levels indicates significant change relevant to the baseline using a non-parametric Wilcoxon matched-pairs signed rank test. Dotted lines indicate mean baseline frequencies of cell subsets in animals allocated to each treatment group.

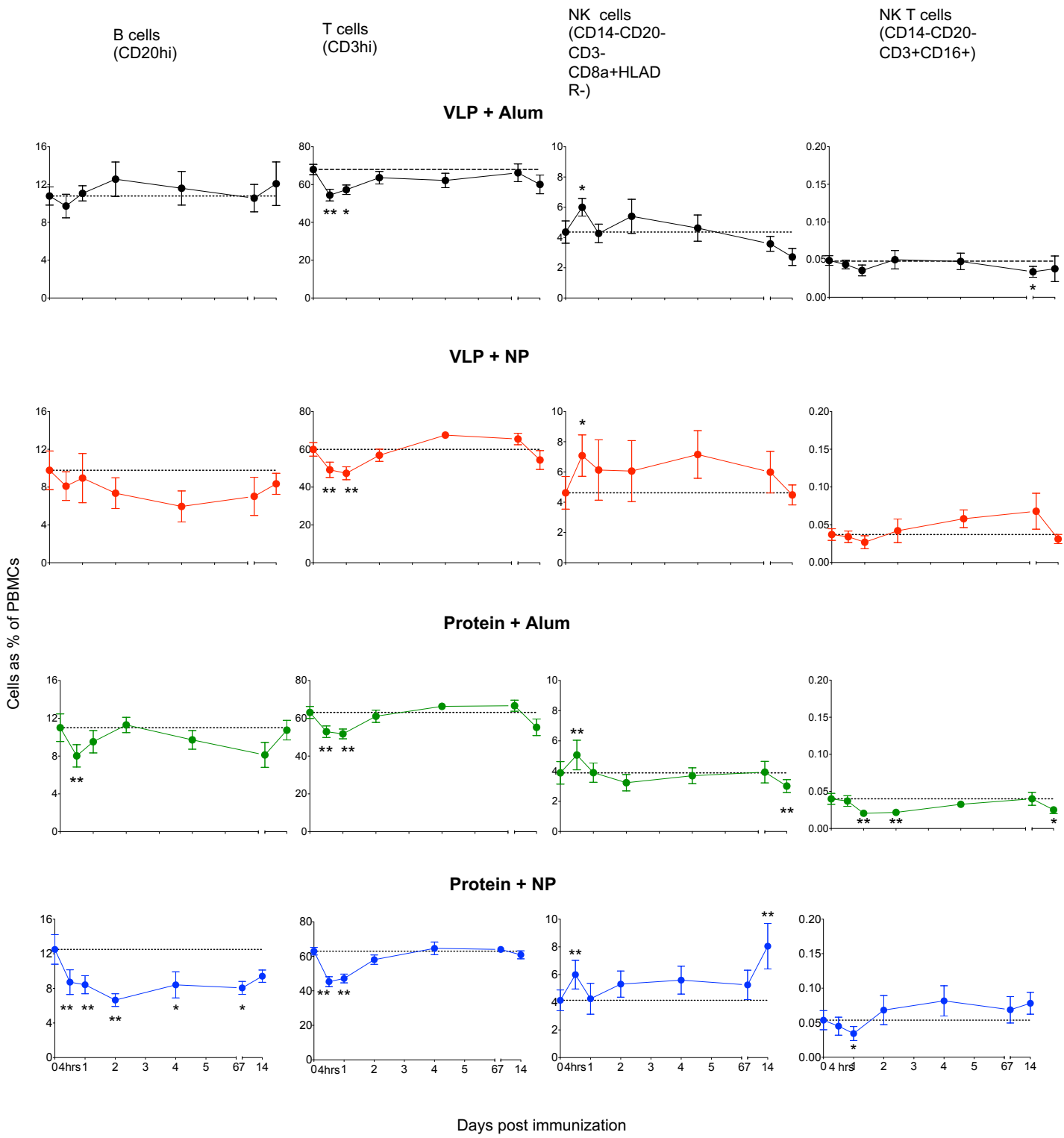
Supplementary Figure 12



Supplementary Figure 12:
CD86 expression on DC subsets in peripheral blood

Graphs indicate changes in expression levels of surface CD86 on DC subsets in peripheral blood after primary immunization. Expressional levels are represented as geometric mean fluorescent intensity (Δ GEO MFI = geometric mean fluorescent signal with anti CD86 antibody – geometric mean fluorescent signal with isotype stain) Statistical significance of changes in expression levels indicates significant change relevant to the baseline using a non-parametric Wilcoxon matched-pairs signed rank test. Dotted lines indicate mean baseline frequencies of cell subsets in animals allocated to each treatment group.

Supplementary Figure 13



Supplementary Figure 13:
Changes in cell subsets in peripheral blood post primary immunization
Line graphs indicate changes in B cells, T cells, NK cells and NK t cells subsets in peripheral blood after primary immunization. Colored dotted lines indicate mean baseline frequencies of cell subsets in animals allocated to each treatment group. Statistical significance of change in frequencies of cell subsets in comparison with baseline was performed using a non-parametric Wilcoxon matched-pairs signed rank test. Dotted lines indicate mean baseline frequencies of cell subsets in animals allocated to each treatment group.